The effective ligase activity was determined by comparing the rate of circularization of EcoRI linerized pMS19 in the solvent above compared with ligase buffer, where 50% of the molecules are circularized in 25 minutes at 100 U/ml. The ligase activity determined was approximately 0.5 U/ml.

Figure 7 depicts the a fluorogram of the branch migration experiments. After 30 minutes, 50% of the recipient duplexes have captured a linker in the presence of BT-D-MedC-1. The rate of linker capture with BT-D-MedC-1 was greater than thirty times that predicted for the ligase activity and the concentrations of displacer-linker duplex using the method in Example 8.

The gel was dried for autoradiography. The branch migration products visible in the fluorogram in lanes 4-6 were visible in the autoradiogram, demonstrating that the new fluorogram bands represent branch migration-mediated capture products.

After overnight incubation, branch migration products could be seen with both displacers with 50% yield of ligated product with BO-D-MedC-1. Thus, the inclusion of a particular, unmodified, triplex-forming region on displacer BT-D-MedC-1, but not BO-D-MedC-1, stabilized the branch migration intermediates by greater than 30-fold.

Although the foregoing invention has been described in some detail by way of illustration and example for clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the following claims.

CLAIMS

We claim:

1. In a method of forming a non stable complex between one strand of a recipient polydeoxynucleotide sequence and a displacer sequence of single stranded DNA where the displacer sequence is at least partially

complementary to such strand of a recipient polydeoxynucleotide sequence, the improvement comprising stabilizing the complex.

2. The method of claim 1 wherein the nonstable complex is stabilized by the presence of at least one modified nucleotide in the displacer strand.

3. The method of claim 1 wherein the complex is stabilized by forming a DNA triplex between the displacer sequence and the recipient duplex.

4. The method of claim 1 wherein the displacer strand comprises a nucleotide sequence and a sequence specific DNA binding moiety that does not significantly melt the recipient DNA duplex at the site to which it attaches.

5. The method of claim 1 wherein the displacer sequence is duplexed with a linker in a displacer-linker duplex wherein the displacer-linker duplex comprises two strands;

1. a displacer strand of which a portion comprises nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex and a portion which comprises a sequence complementary to and hybridized with a linker strand, and

2. a linker strand complementary to and hybridized with the displacer strand.

6. An oligo- or polydeoxynucleotide displacer-linker duplex which is capable of initiating branch migration at the end of a recipient polydeoxynucleotide duplex without the prior formation of a stable hybrid with such recipient polydeoxynucleotide duplex, which displacer-linker duplex comprises two strands;

a. a displacer strand of which a portion comprises nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex and a portion which comprises a sequence complementary to and hybridized with a linker strand, and

b. a linker strand complementary to and hybridized with the displacer strand.

7. The displacer-linker duplex of claim 6 which can initiate branch migration at a restriction endonuclease cleavage site.

8. The displacer-linker duplex of claim 6 which can hybridize to and initiate branch migration adjacent to a 3' or 5' single stranded extension on the recipient polydeoxynucleotide duplex.

9. The displacer-linker duplex of claim 6 wherein at least one of the nucleotides complementary to one strand of the recipient polydeoxynucleotide duplex is modified to increase the stability of the hybrid displacer-recipient duplex.

10. The displacer-linker duplex of claim 6 wherein at least one of the nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex is modified to increase the melting temperature of the hybrid displacer-recipient duplex.

11. The displacer-linker duplex of claim 9 wherein the modified nucleotide is selected from the group consisting of modified nucleotides which increase the association constant with the complementary deoxynucleotide by at least about 20 percent.

12. The displacer-linker duplex of claim 9 wherein the modified nucleotide is selected from the group consisting of 5-halogenated pyrimidine nucleotides, 5-methyldeoxycytidine, diaminopurine deoxynucleotide, ribonucleotides, and 2'-alkylated ribonucleotides.

13. The displacer-linker duplex of claim 9 wherein the modified nucleotide is a 5-halogenated pyrimidine nucleotide.

14. The displacer-linker duplex of claim 9 wherein the modified nucleotide is 5-bromodeoxyuridine.

15. The displacer-linker duplex of claim 9 wherein the modified nucleotide is 5-methyldeoxycytidine.

16. The displacer-linker duplex of claim 6 which contains a modification which permits detection of the displacer-recipient hybrid.

17. The modified displacer-linker duplex of claim 16 wherein the modification is selected from the group consisting of radioactive labels, fluorescent labels and targets for detection, including biotin moieties, enzymes and phosphorothioate linkages.

18. The modified displacer-linker duplex of claim 16 wherein the modification is present in the linker.

19. The displacer-linker duplex of claim 6 which contains a modification which allows capture of the displacer-recipient hybrid by affinity chromatography.

20. The displacer-linker duplex of claim 19 wherein the modification is selected from the group consisting of biotin moieties and phosphorothioate linkages.

21. The displacer-linker duplex of claim 19 wherein the modification is present in the linker.

22. The displacer-linker duplex of claim 6 which also comprises a 5' or 3' single-stranded extension complementary to a 5' or 3' single-stranded extension resulting from the digestion of a polydeoxynucleotide duplex with a restriction endonuclease.

23. An artificially constructed polydeoxynucleotide hybrid comprising a naturally occurring recipient polydeoxynucleotide duplex hybridized to the displacer-linker duplex of any of claims 6-22.

24. The artificially constructed polydeoxynucleotide hybrid of any of claims 6-22 wherein the linker strand is covalently linked to one of the strands of the recipient duplex.

25. An artificially constructed polydeoxynucleotide hybrid comprising a naturally occurring recipient polydeoxynucleotide duplex hybridized to the displacer of claims 57-87.

26. A method of modifying a recipient polydeoxynucleotide duplex by contacting such recipient polydeoxynucleotide duplex with a displacer-linker duplex under conditions that permit the formation of a hybrid polydeoxynucleotide duplex, wherein

the displacer-linker duplex comprises two strands;

1. a displacer strand of which a portion comprises nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex and a portion which comprises a sequence complementary to and hybridized with a linker strand, and

2. a linker strand complementary to and hybridized with the displacer strand.

27. The method of claim 26 wherein the recipient duplex terminates in a 3' or 5' single stranded extension and the displacer strand contains a sequence complementary to the extension.

28. The method of claim 26 where the hybrid is stabilized after formation of the hybrid polynucleotide duplex.

29. The method of claim 28 wherein the hybrid is stabilized by covalently linking the linker strand of the displacer-linker duplex to the strand of the recipient duplex complementary to the displacer strand.

30. The method of claim 29 wherein the covalent link is a phosphodiester linkage.

31. The method of claim 29 wherein the hybrid is stabilized by ligating the linker strand of the displacer-linker duplex to the strand of the recipient duplex complementary to the displacer strand using T4 DNA ligase.

32. The method of claim 26 wherein at least one of the nucleotides complementary to one strand of the recipient polydeoxynucleotide duplex is a modified nucleotide which increases the stability of the hybrid displacer-recipient duplex.

33. The method of claim 26 wherein at least one of the nucleotides complementary to one strand of the recipient polydeoxynucleotide duplex is a modified nucleotide which increases the melting temperature of the hybrid displacer-recipient duplex.

34. The method of claim 32 wherein the modified nucleotide is selected from the group consisting of modified nucleotides which increase the association constant with the complementary deoxynucleotide by at least about 20 percent.

35. The method of claim 32 wherein the modified nucleotide is selected from the group consisting of 5-halogenated pyrimidine nucleotides, 5-methyldeoxycytidine, diaminopurine deoxynucleotide, ribonucleotides, and 2'-alkylated ribonucleotides.

36. The method of claim 32 wherein the modified nucleotide is a 5-halogenated pyrimidine nucleotide.

37. The method of claim 32 wherein the modified nucleotide is 5-bromodeoxycytidine.

38. The method of claim 32 wherein the modified nucleotide is 5-methyldeoxycytidine.

39. The method of claim 32 wherein from about 10 percent to about 80 percent of the nucleotides complementary to one strand of the recipient polydeoxynucleotide duplex are modified nucleotides.

40. A method of labelling an artificially constructed nucleic acid hybrid of a naturally occurring recipient polydeoxynucleotide duplex hybridized to a displacer-linker duplex which is capable of initiating branch migration at the end of the recipient polydeoxynucleotide duplex without the prior formation of a stable hybrid with such recipient polydeoxynucleotide duplex, which displacer-linker duplex comprises two strands;

1. a displacer strand of which a portion comprises nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex and a portion which comprises a sequence complementary to and hybridized with a linker strand, and

2. a linker strand complementary to and hybridized with the displacer strand,

which method of labelling comprises modifying the displacer-linker duplex to incorporate therein a modification which permits detection of the artificially constructed nucleic acid hybrid.

41. The method of claim 40 wherein the displacer-linker duplex is modified prior to hybridization with the naturally occurring recipient polydeoxynucleotide duplex.

42. The method of claim 40 wherein the linker strand of the displacer-linker duplex is covalently linked to the strand of the naturally occurring recipient polydeoxynucleotide duplex complementary to the displacer strand.

43. The method of claim 40 wherein the modification is selected from the group consisting of radioactive labels, fluorescent labels, enzymes and chemical labels including biotin moieties and phosphorothioate linkages.

44. The method of claim 40 wherein the modification is selected from the group consisting of targets for affinity chromatography.

45. The method of claim 44 wherein the modification is selected from the group consisting of biotin moieties and phosphorothioate linkages.

46. The method of claim 40 wherein the modification comprises a 5' or 3' single-stranded extension of the displacer-linker duplex, which extension

1) is unaffected by formation of the displacer-linker-recipient polydeoxynucleotide duplex hybrid, and

2) is a target for attachment to polydeoxynucleotide duplexes containing complementary 5' or 3' single stranded extensions.

47. The method of claim 40 used to label one end of a cloned deoxynucleotide insert in a vector.

48. The method of claim 47 where the vector is a plasmid vector.

49. The method of claim 47 where the vector is a cosmid vector.

50. The method of claim 47 where the vector is a yeast artificial chromosome vector.

51. In a method of restriction endonuclease mapping of an insert, the improvement comprising labelling the insert by the method of claim 40.

52. In a method of capture of an artificially constructed nucleic acid hybrid by affinity chromatography, the improvement comprising labelling the hybrid by the method of claim 40.

53. In a method of enriching a recipient polydeoxynucleotide duplex in a population of polydeoxynucleotide duplexes, the improvement

comprising labelling the recipient polydeoxynucleotide duplex by the method of claim 40.

54. In a method of covalently attaching a restriction endonuclease linker onto a recipient polydeoxynucleotide duplex, the improvement comprising labelling the resulting hybrid by the method of claim 40.

55. In a method of selectively cloning a recipient polydeoxynucleotide duplex by covalently attaching a restriction endonuclease linker onto such recipient polydeoxynucleotide duplex, the improvement comprising labelling the hybrid by the method of claim 40.

56. In a method of isolating clones of contiguous polydeoxyribonucleotide duplexes by covalently attaching a restriction endonuclease linker onto the duplexes, the improvement comprising labelling the clones by the method of claim 40.

57. An oligo- or polydeoxynucleotide displacer which is capable of binding to a recipient polydeoxynucleotide duplex which displacer comprises

1) a first sequence which is capable of initiating triple helix formation, and which comprises

a) at least six consecutive pyrimidine bases or

b) at least seven bases where at least six of the bases are pyrimidine bases and the seventh base is guanine, and

2) a second sequence proximate to such first sequence which is complementary to and runs antiparallel to the second strand of the recipient duplex and which is capable of initiating branch migration proximate to the triple helix.

58. The displacer of claim 57 wherein the second sequence is adjacent to the first sequence.

59. The displacer of claim 57 wherein the second sequence is separated from the first sequence by from 1 to 5 intervening moieties.

60. The displacer of claim 59 wherein the intervening moieties are nucleotides.

61. The displacer of claim 60 wherein at least one of the moieties is a modified nucleotide.

62. The displacer of claim 59 wherein wherein one of the intervening moieties has an intercalating agent covalently attached.

63. The displacer of claim 57 wherein at least one of the nucleotides complementary to one strand of the recipient polydeoxynucleotide duplex is modified to increase the stability of the displacer-recipient complex.

64. The displacer of claim 57 wherein at least one of the nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex is modified to increase the melting temperature of the displacer-recipient complex.

65. The displacer of claim 63 wherein the modified nucleotide is selected from the group consisting of modified nucleotides which increase the association constant with the complementary deoxynucleotide by at least about 20 percent.

66. The displacer of claim 63 wherein the modification is in the first sequence.

67. The displacer of claim 66 wherein the modified nucleotide is a 5-halogenated pyrimidine nucleotide.

68. The displacer of claim 66 wherein the modified nucleotide is selected from the group consisting of 5-bromodeoxyuridine and 5-methyldeoxycytidine.

69. The displacer of claim 63 wherein the modification is in the second sequence.

70. The displacer of claim 69 wherein the modified nucleotide is selected from the group consisting of 5-halogenated pyrimidine nucleotides, 5-methyldeoxycytidine, diaminopurine deoxynucleotide, ribonucleotides and a 2'-alkylated ribonucleotides.

71. The displacer of claim 69 wherein the modified nucleotide is a 5-halogenated pyrimidine nucleotide.

72. The displacer of claim 69 wherein the modified nucleotide is 5-bromodeoxyuridine.

73. The displacer of claim 69 wherein the modified nucleotide is 5-methyldeoxycytidine.

74. The displacer of claim 57 which further comprises at least one moiety attached to a terminus of the oligo or polydeoxynucleotide, which moiety confers endonuclease resistance to the terminus to which it is attached.

75. The displacer of claim 74 wherein the moiety is attached to the deoxyribose moiety of a terminal nucleotide.

76. The displacer of claim 75 wherein the moiety is indirectly attached to the deoxyribose moiety of a terminal nucleotide.

77. The displacer of claim 74 wherein the moiety is attached to the hydroxyl group of a terminal nucleotide.

78. The displacer of claim 74 wherein the moiety is attached to the phosphate moiety of a terminal nucleotide.

79. The displacer of claim 74 where the moiety is selected from the group consisting of intercalating agents, isoureas, carbodiimides and N-hydroxybenzotriazoles.

80. The displacer of claim 77 wherein the moiety is a methylthiophosphonate.

81. The displacer of claim 74 wherein the moiety is a selected from the group consisting of polypeptides and proteins.

82. The displacer of claim 74 wherein the moiety is a 2',3'-dideoxyribose nucleotide attached to the 3'-terminal deoxyribonucleotide through a phosphodiester linkage.

83. The displacer of claim 82 wherein the 2',3'-dideoxyribose nucleotide is a modified 2',3'-dideoxyribose nucleotide.

84. The displacer of claim 57 which contains a modification which permits detection of the displacer-recipient hybrid.

85. The modified displacer of claim 84 wherein the modification is selected from the group consisting of radioactive labels, fluorescent labels, enzymes and targets for detection, including biotin moieties and phosphorothioate linkages.

86. The displacer of claim 57 which contains a modification which allows capture of the displacer-recipient hybrid by affinity chromatography.

87. The displacer of claim 86 wherein the modification is selected from the group consisting of biotin moieties and phosphorothioate linkages.

88. An method of modifying a recipient polydeoxynucleotide duplex comprising contacting such recipient polydeoxynucleotide duplex with the displacer of claim 57 under conditions that permit the formation of a complex.

89. The method of claim 88 wherein at least one of the nucleotides complementary to one strand of the recipient polydeoxynucleotide

duplex is modified to increase the stability of the displacer-recipient complex.

90. The method of claim 88 wherein at least one of the nucleotides complementary to one strand of a recipient polydeoxynucleotide duplex is modified to increase the melting temperature of the displacer-recipient complex.

91. The method of claim 88 wherein the modified nucleotide is selected from the group consisting of modified nucleotides which increase the association constant with the complementary deoxynucleotide by at least about 20 percent.

92. The method of claim 88 wherein the modification is in the first sequence of the displacer of claim 57.

93. The method of claim 92 wherein the modified nucleotide is selected from the group consisting of 5-bromodeoxyuridine.and 5-methyldeoxycytidine.

94. The method of claim 88 wherein the modification is in the second sequence of the displacer of claim 57.

95. The method of claim 94 wherein the modified bases are selected from the group consisting of 5-halogenated pyrimidine nucleotides, 5-methyldeoxycytidine, diaminopurine deoxynucleotide, ribonucleotides, and 2'-alkylated ribonucleotides.

96. The method of claim 94 wherein the modified nucleotide is a 5-halogenated pyrimidine nucleotide.

97. The method of claim 94 wherein the modified nucleotide is 5-bromodeoxyuridine.

98. The method of claim 94 wherein the modified nucleotide is 5-methyldeoxycytidine.

99. The method of claim 88 wherein the displacer contains at least one moiety attached to a terminus of the oligo or polynucleotide, which moiety confers endonuclease resistance to the terminus to which it is attached.

100. The method of claim 99 wherein the moiety is attached to the deoxyribose moiety of a terminal nucleotide.

101. The method of claim 100 wherein the moiety is indirectly attached to the deoxyribose moiety of a terminal nucleotide.

102. The method of claim 99 wherein the moiety is attached to the hydroxyl group of a terminal nucleotide.

103. The method of claim 99 wherein the moiety is attached to the phosphate moiety of a terminal nucleotide.

104. The method of claim 99 where the moiety is selected from the group consisting of intercalating agents, isoureas, carbodiimides and N-hydroxybenzotriazoles.

105. The method of claim 101 wherein the moiety is a methylthiophosphonate.

106 The method of claim 99 wherein the moiety is a selected from the group consisting of polypeptides and proteins.

107. The method of claim 99 wherein the moiety is a 2',3'-dideoxyribose nucleotide attached to the 3'-terminal deoxyribonucleotide through a phosphodiester linkage.

108. The method of claim 107 wherein the 2',3'-dideoxyribose nucleotide is a modified 2',3'-dideoxyribose nucleotide.

109. A method of labelling a displacer-recipient complex comprising contacting a recipient polydeoxynucleotide duplex with the displacer of claim 101 under conditions that permit the formation of a complex wherein the displacer contains a modification which will permit detection of the displacer-recipient complex.

110. The method of claim 109 wherein the modification is selected from the group consisting of radioactive labels, fluorescent and chemiluminescent labels, enzymes and targets for detection.

111. The method of claim 109 wherein the modification is selected from the group consisting of targets for affinity chromatography.

112. The method of claim 111 wherein the modification is selected from the group consisting of biotin moieties and phosphorothioate linkages.

113. In a method of capture of an artificially constructed nucleic acid hybrid by affinity chromatography, the improvement comprising modifying the hybrid by the method of claim 88.

114. In a method of enriching a recipient polydeoxynucleotide duplex in a population of polydeoxynucleotide duplexes, the improvement comprising labelling the recipient polydeoxynucleotide duplex by the method of claim 88.

115. In a method for the site specific addition, deletion or alteration of nucleotides in a recipient polydeoxynucleotide duplex, the improvement comprising modifying the duplex by the method of claim 88.

116. In a method of repairing a mutational lesion comprising replacing a naturally occurring strand of DNA with a modified strand of DNA, the improvement wherein the new strand is introduced to the naturally occurring duplex by the method of claim 88 and displaces the original strand.